Hepatic Xanthine Oxidase and Ferritin Iron in the Developing Rat

By A. MAzUR AND A. CARLETON

The results of studies in our laboratory lend support to the hypothesis that the release of iron from ferritin stores in the liver is mediated by the enzyme xanthine oxidase acting as a dehydrogenase. In this reaction the reduced enzyme, formed as a result of oxidation of xanthine or hypoxanthine to uric acid, is reoxidized by some of the ferric iron of ferritin which is therefore reduced to the ferrous state. Reduced ferritin iron is less tightly bound to the protein than is the ferric form and dissociates easily in the presence of an avid iron acceptor such as the serum iron-binding protein transferrin.

Since the newborn animal is dependent for its dietary iron on mother's milk which is deficient in this element, it has been presumed that iron required by the preweanling animal for purposes of hemoglobin synthesis originates from iron stored during fetal life. Westerfeld and Richert reported the virtual absence of liver xanthine oxidase in newborn rats, and early studies suggested the absence of this enzyme in liver of newborn human infants. If xanthine oxidase is responsible for the release of iron from hepatic ferritin at a rate greater than that which would normally occur as a result of protein turnover, xanthine oxidase must appear in the liver at a time coincident with the release of liver ferritin iron during the course of animal development.

Results of the present study confirm an inverse relationship between ferritin iron content and xanthine oxidase activity in liver of the developing rat.

Methods

Rats used in this study were of the CFN strain, Carworth Farms, Inc., New City, New York. In vitro studies with liver were performed using mature female rats, whereas those done with fetal, newborn or weanling rats utilized liver from all rats of the same litter.

Xanthine oxidase activity was determined by the method of Westerfeld and Richert and were confirmed in many cases by the microphotofluorometric method of Burch et al. using 5 x 10^{-6} M methylene blue. For liver, an aliquot of total liver homogenate was used for analyses, whereas for intestinal mucosa the first 10 cm. of adult rat intestine, measured from the pylorus, or a comparable section from younger rats, was homogenized. Uricase was determined by the substitution of uric acid as substrate in place of hypoxanthine in the method of Westerfeld and Richert.

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Fig. 1.—Hepatic ferritin iron (μg. Fe per Gm. liver dry weight) hepatic xanthine oxidase and intestinal xanthine oxidase in the developing rat (μl. O₂ per Gm. dry weight per hour.). Each value represents the mean and standard error for 10–12 rats.

Ferritin was isolated for quantitative estimation by precipitation with rabbit antihorse ferritin serum which cross-reacts with rat ferritin and, in the presence of sufficient antiserum, precipitates rat ferritin quantitatively. The washed specific precipitate was analyzed for total Fe and total N.

To follow the fate of ferritin iron in incubating liver slices, they were preincubated for 30 minutes in O₂ with rat serum-bound Fe⁵⁹. the slices washed with Krebs-Ringer-phosphate medium and reincubated in this medium together with unlabeled rat serum. Similarly, to follow the fate of the protein moiety of ferritin, an aliquot of liver slices was preincubated for 30 minutes in O₂ with 2-C¹⁴-glycine, washed with medium and reincubated in the medium together with unlabeled glycine. Ferritin was isolated in each case by precipitation with its antibody and aliquots assayed for radioactivity: Fe⁵⁹ in a crystal scintillation counter and C¹⁴ in a windowless gas flow counter.

Analyses of liver slices for hypoxanthine + xanthine, uric acid and allantoin were performed by methods previously described.¹

RESULTS

Figure 1 illustrates the relationship between liver ferritin iron and xanthine oxidase activity in the developing rat. The newborn rat liver contains a relatively larger quantity of ferritin iron, per gram of liver, as compared with mature rat liver, and accumulates during fetal life. On the other hand, xanthine oxidase activity is very low or absent in fetal or newborn rat liver, confirming the findings of Westerfield.³

Soon after birth (6–10 days) the liver ferritin content falls coincident with the appearance of xanthine oxidase. At weaning (21 days), ferritin iron stores in the liver are markedly depleted, but after ingestion of a normal diet,
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Table 1.—Iron Content of Liver Ferritin in the Developing Rat

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Liver Weight</th>
<th>Ferritin Iron in Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>g.</td>
<td>g.</td>
<td>per g. µg.</td>
</tr>
<tr>
<td>4.0</td>
<td>0.25 ± 0.01</td>
<td>480 ± 20</td>
</tr>
<tr>
<td>6.0</td>
<td>0.27 ± 0.01</td>
<td>597 ± 25</td>
</tr>
<tr>
<td>9.6</td>
<td>0.35 ± 0.01</td>
<td>361 ± 22</td>
</tr>
<tr>
<td>38</td>
<td>1.48 ± 0.17</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>90</td>
<td>3.89 ± 0.17</td>
<td>140 ± 11</td>
</tr>
<tr>
<td>149</td>
<td>6.17 ± 0.18</td>
<td>243 ± 39</td>
</tr>
<tr>
<td>228</td>
<td>8.26 ± 0.47</td>
<td>431 ± 30</td>
</tr>
<tr>
<td>270 (Mothers)</td>
<td>11.0 ± 1.4</td>
<td>76 ± 17</td>
</tr>
</tbody>
</table>

Each value is the mean ± standard error for 10 to 12 rats per sample, calculated for dry weight of liver.

Ferritin iron gradually accumulates in the liver reaching normal values at maturity. During this period, xanthine oxidase activity in the liver increases to adult levels. It is of interest to note that maternal iron stores in the liver are markedly depleted, intestinal xanthine oxidase activity is present in substantial quantities in both the fetal and newborn rat in contrast with the virtual absence of the enzyme in liver, and uricase, the enzyme in rat liver which converts uric acid to allantoin, can be demonstrated at almost adult levels in the newborn rat.

Table 1 shows the values of ferritin iron calculated per grain of liver as well as for total liver. In either case the results demonstrate a marked decrease in stored ferritin iron after birth followed by an increase in accumulated iron after weaning.

Absence of an enzymatic release mechanism for ferritin iron in the liver of the newborn rat in contrast to its presence in the liver of adult rats is confirmed by the results of in vitro isotopic-labeling experiments. The results of one experiment are shown in figure 2. Similar results were obtained in four such experiments. Whereas ferritin in surviving liver slices from adult rats, labeled by preincubation with serum-bound Fe\(^{59}\) or with 2-C\(^{14}\) glycine, loses its Fe\(^{59}\) much faster than its C\(^{14}\) after subsequent incubation in a nonisotopic medium, ferritin from liver of newborn rats treated in an identical manner shows little change either in Fe\(^{59}\) or C\(^{14}\). These results suggest that the release of ferritin iron from the liver of adult rats occurs by a mechanism much faster than, and independent of, protein degradation, whereas any release of ferritin iron from the liver of newborn or fetal rats occurs only as a result of, and at the normal rate of protein turnover.

Additional confirmation of these findings was obtained by direct measurement of the formation or disappearance of hypoxanthine (+xanthine), and allantoin after incubation of liver slices from fetal, newborn, weanling and adult rats. Table 2 demonstrates that although there is a disappearance of hypoxanthine in liver slices from weanling and adult rats, it accumulates in liver slices taken from fetal or newborn rats. Whereas considerable allantoin accumulates in slices from weanling and adult rats, reflecting the adequate
**Fig. 2.** — Release of Fe⁵⁹ or C¹⁴ from liver slices of (a) newborn rats (b) adult rats. One Gm. liver slices was preincubated either with serum-bound Fe⁵⁹ or 2-C¹⁴-glycine in 10 ml. of Ringer-phosphate for 30 minutes, washed free of excess isotope and reincubated with nonisotopic serum or glycine, respectively. Radioactivity is expressed as specific activity of Fe⁵⁹, adjusted to a value of 100 for zero time of preincubation.

formation of uric acid, little allantoin is formed in incubated liver taken from fetal and newborn rats, confirming the very low rate of uric acid formation.

**DISCUSSION**

These findings help to clarify the mechanism by which the fetal rat is able to store iron derived from maternal serum, since the absence of xanthine oxidase in the fetal liver restricts its release to the normal process of ferritin protein turnover. Soon after birth, during a time when the need for iron for hemoglobin synthesis is increasing rapidly and the maternal source of iron is gone, xanthine oxidase appears in the liver and ferritin iron is now released into the plasma from which it may be sequestered by the marrow for hemoglobin synthesis. At weaning, when the reserve ferritin iron of the liver has been almost completely depleted, the rat turns to a normal diet for its iron, absorbing enough of this element for hemoglobin synthesis...
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Table 2.—Purine Metabolism in Rat Liver Slices at Various Stages of Development

<table>
<thead>
<tr>
<th>Age of Rat</th>
<th>Increase or Decrease of</th>
<th>Hypoxanthine</th>
<th>Allantoin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus (−2 days)</td>
<td>Increase</td>
<td>+1.23</td>
<td>+1.32</td>
</tr>
<tr>
<td></td>
<td>(1.16–1.40)</td>
<td>(1.01–1.55)</td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>Increase</td>
<td>+0.59</td>
<td>+1.30</td>
</tr>
<tr>
<td></td>
<td>(0.25–0.83)</td>
<td>(1.10–1.59)</td>
<td></td>
</tr>
<tr>
<td>Weanling (21 days)</td>
<td>Decrease</td>
<td>−1.07</td>
<td>+10.24</td>
</tr>
<tr>
<td></td>
<td>(0.85–1.25)</td>
<td>(8.21–12.50)</td>
<td></td>
</tr>
<tr>
<td>Adult (100 days)</td>
<td>Decrease</td>
<td>−1.26</td>
<td>+6.10</td>
</tr>
<tr>
<td></td>
<td>(1.08–1.45)</td>
<td>(5.10–8.50)</td>
<td></td>
</tr>
</tbody>
</table>

One Gm. of liver slices was suspended in 10 ml Krebs-Ringer-phosphate medium, pH 7.4 and incubated for 1 hr. in oxygen. These and control nonincubated samples were analysed for hypoxanthine (+ xanthine) and allantoin. The results are expressed as mg. per g. of liver protein per hour. Values are the mean for 6–10 rats in each group; numbers in parenthesis show the spread.

as well as for storage as hepatic ferritin. From these results it seems apparent why an animal which continues on a diet restricted to mother’s milk will soon become anemic due to iron deficiency. The abnormally low ferritin iron stores in the liver of the mother rats at parturition emphasizes the extent of diversion of such iron to the fetus.

SUMMARY

The absence of hepatic xanthine oxidase in the fetus and newborn rat is associated with a very high liver ferritin iron content. Soon after birth hepatic xanthine oxidase activity increases significantly coincident with a marked decrease in liver ferritin iron content. At weaning, hepatic ferritin iron is very low but slowly rises subsequent to intake of a normal diet containing iron.

SUMMARIO IN INTERLINGUA

Le absentia de oxydase de xanthina in le hepate de fetal e neonate rattos es associate con un altissime contento hepatic de ferro de ferritina. Tosto post nato le activitate de oxidase de xanthina in le hepate accresce significativemente in coincidentia con un declino marcate in le contento hepatic de ferro de ferritina. Al tempore del dislactamento, le contento hepatic de ferro de ferritina es bassissime, sed subsequentemente illo monta secundari al ingestion de un dieta normal a contento de ferro.

REFERENCES

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